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Assessing the Movement of *Cucurbit yellow stunting disorder virus* in Susceptible and Tolerant Cucumber Germplasms Using Serological and Nucleic Acid-based Methods

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Abstract

Cucurbit yellow stunting disorder virus (CYSDV) is an emerging virus causing significant yield losses in cucurbits. Simple but reliable detection and quantification methods are important tools for disease management. In susceptible germplasm, CYSDV was detected 5 days postinoculation (dpi) by reverse transcriptase polymerase chain reaction (RT-PCR) or by tissue blot immunoassay (TBIA), and 8–9 dpi by dot blot immunoassay (DBIA) or enzyme-linked immunosorbent assay (ELISA). For CYSDV quantification, real-time RT-PCR was the most sensitive method and gave the best linear range of detection, over four orders of magnitude as compared to approximately two orders of magnitude for DBIA and nucleic acid hybridization. DBIA was more sensitive than ELISA and equally sensitive to nucleic acid hybridization with a non-radioactively labelled cDNA probe. Time course studies at 3, 5, 8 and 14 dpi using TBIA revealed that tolerance to CYSDV in three tolerant cucumber germplasms was not correlated with restricted or delayed virus movement.

Introduction

Cucurbit yellow stunting disorder virus (CYSDV) is a member of the genus *Crinivirus* (family *Closteroviridae*) efficiently transmitted by *Bemisia tabaci*, biotypes 'B' (*B. argentifolii*) and 'Q' (Berdiales et al., 1999). The natural host range of CYSDV is limited to the family Cucurbitaceae including important crops, such as cucumber, melon, watermelon and squash. The severe yellowing induced by CYSDV drastically affects fruit number and weight leading to a 30–50% yield reduction (Abou-Jawdah et al., 2000; López-Sése and Gomez-Guillamon, 2000). CYSDV was first reported in the United Arab Emirates in 1991, but may have caused cucurbit diseases in the Mediterranean region since the early 1980s (Hassan and Duffus, 1991). CYSDV has spread rapidly in the Arabic Peninsula, Eastern and Western Mediterranean basin Egypt, Israel, Jordan, Lebanon, Syria and Saudi Arabia (Duffus, 1995; Abou-Jawdah et al., 2000), Spain (Célix et al., 1996), Morocco (Desbiez et al., 2000), Portugal (Louro et al., 2000), France (Pacheco, 2005) and North America (Kao et al., 2000).

CYSDV is considered a plant quarantine pest in several regions (Pacheco, 2005). Fast, simple, reliable and sensitive detection and quantification methods are required for monitoring of virus spread and host range, for determination of virus titre in resistant, tolerant or susceptible cucurbit genotypes, and for determination of virus load in insect vectors. Due to the labile nature of CYSDV particles, and the subsequent difficulty in development of serological tests, detection of CYSDV has relied for over a decade on nucleic acid hybridization and reverse transcriptase polymerase chain reaction (RT-PCR). For CYSDV quantification, nucleic acid hybridization using chemiluminescent detection (CDP) system was predominantly used as it provides sensitive detection and is less hazardous than radioactive methods (Ruiz et al., 2002; Marco et al., 2003). Recently CYSDV coat protein (CP) was expressed in a bacterial expression system and used to develop CYSDV-specific antibodies that were used in serological assays to detect CYSDV (Hourani and Abou-Jawdah, 2003; Cotillon et al., 2005).

Recently 124 cucumber accessions were evaluated for resistance against CYSDV. No immune or highly resistant accessions were detected, but some accessions (PI 605923, PI 293432 and PI 211589) were tolerant and showed delayed expression of symptoms, milder final symptoms, and lower percentages of infected plants compared with susceptible cucumber (Eid et al., 2006). Several mechanisms for virus tolerance have been reported, including resistance to virus movement between cells, resistance to long distance movement of

virus within the plant and resistance to virus multiplication or to virus accumulation (Lecoq et al., 2004). We have investigated whether CYSDV tolerance in cucumber germplasms is correlated with delayed virus movement or accumulation, and evaluated the relative sensitivity of CYSDV serological detection methods as compared with nucleic acid-based methods.

Materials and Methods

Source of CYSDV infected tissue

A CYSDV isolate collected from a CYSDV-infected cucumber plant grown in a greenhouse in North Lebanon was maintained in CYSDV-infected cucumber plants grown in 30-cm diameter plastic pots in insect-proof glasshouse equipped with ventilation when temperature exceeds 30°C. New seedlings were periodically inoculated using whitefly transmission every 45–60 days. Leaf tissue samples were collected from plants showing prominent inter-veinal yellowing but before development of widespread yellowing.

CYSDV detection by serological methods

Tissue blot immunoassay

Leaf midribs and/or petioles of CYSDV-infected and healthy cucumber plants were cut with a razor blade and immediately the pieces were blotted for 20 s onto positively charged Nylon membrane (Amersham Biosciences, Little Chalfont, Bucks, UK). Membrane blocking, application of primary and secondary antibodies and colorimetric detection methods were performed as described previously (Hourani and Abou-Jawdah, 2003). The dried membranes were examined under a Stemi DV4 Stereoscope (Carl Zeiss, Berlin, Germany) for colour precipitation in phloem cells where the virus is located.

Dot blot immunoassays

Leaf samples consisting of 1 g of leaf midrib were homogenized in 20 ml tris buffer saline pH 8.0 + 0.1% tween 20 (TBST) + 0.1% sodium sulphite. Nylon membranes were soaked in distilled water for 10 min and then in extraction buffer for 1 min. After washing each well with 100 μl of TBST buffer; the sample extracts (6 μl of each leaf extract dilution + 80 μl TBST) were applied under vacuum in the wells of the Bio-dot apparatus (Bio-Rad®, Hercules, CA, USA). Serial dilutions (1 : 9 and 1 : 1) of infected leaf extracts in healthy leaf extracts were blotted on Nylon membranes. The membranes were blocked for 2 h with TBST buffer containing 5% skimmed milk (Regilait®, Saint Martin Belle Roche, France) + 1% bovine serum albumin (Fraction V, reagent grade, BIOREBA AG®, Reinach, Switzerland). CYSDV was immunologically localized using purified CYSDV polyclonal antibodies (1 : 1000) as primary antibodies and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulins (Sigma, St Louis, MO, USA) as secondary antibodies (1 : 10 000) (Hourani and Abou-Jawdah, 2003). For detection, the CDP-star substrate (Amersham Biosciences) was diluted (1/20) and applied to the membranes for 5 min. Thirty minutes after draining off excess detection reagent, the best exposure time of X-ray films (AGFA CP-BU, Medical X-ray film blue; Agfa-Gevaert N.V, Morstel, Belgium) was usually approximately 20 min. To quantify the signal intensity of each dot, autoradiograms were scanned and computer analyzed using the Labworks imaging and analysis software (Ultra-Violet products Ltd., Cambridge, UK), the signal of the healthy control was considered as background reference.

ELISA

Leaf extracts were prepared as described for DBIA. Aliquots of 100 μl leaf extracts and serial dilutions (1 : 1) of CYSDV infected leaf extracts diluted in healthy leaf extracts were applied per well, two replicates per dilution. ELISA was performed as described earlier for plate-trapped antigen (Eid et al., 2006).

CYSDV detection by nucleic acid-based methods

Total RNA extraction and nucleic acid hybridization

Total RNAs were extracted from healthy and CYSDV-infected cucumber leaf tissue using the Tri-Reagent (Trizol® Reagent; Invitrogen, Scotland, UK) as described by Hourani and Abou-Jawdah (2003). The quality of extracted RNAs was checked by electrophoresis in 0.8% agarose gels and quantity which varied from 2.8 to 8.0 μg/μl was measured using Gene Quant photometer (Amersham Biosciences).

RNA extracts were denatured at 68°C for 5 min then quenched on ice for 5 min. Serial dilutions (1 : 10) of the total RNAs from both healthy and infected tissue were spotted on positively charged Zeta-Probe® GT nylon membranes (Bio-Rad, CA, USA). Aliquots (2 μl of each RNA dilution + 80 μl DNase–RNase-free water) were spotted on membranes using a Bio-dot apparatus (Bio-Rad®) and RNAs were fixed using a UV crosslinker (Spectrolinker XL100 UV crosslinker, Spectronics Corporation, WestBury, New York, USA).

cDNA probes corresponding to CP gene (800 bp) and HSP70gene (460 bp) were labelled with digoxigenin (DIG), using Dig-labelled uridine triphosphate, in RT-PCR according to the manufacturer's recommendations (Roche Diagnostics, Indianapolis, IN, USA). CYSDV RNA spotted on membrane was detected by molecular hybridization with a cDNA probe using DIG High Prime DNA Labelling and Detection Starter Kit II (Roche Diagnostics).

Prehybridization was carried out in DIG Easy Hyb buffer (Roche Diagnostics) for 2 h at 42°C. Denatured Dig-labelled probes were added at 25 ng/ml and the hybridization performed overnight at 42–50°C. Membranes were washed twice for 5 min each in 2 × 0.15M NaCl + 0.015 sodium citrate, pH 7.0 (SSC) and 0.1% sodium dodecyl sulphate (SDS) at room temperature and twice for 15 min each in 0.5 × SSC and 0.1% SDS at 65°C. CDP was carried out using the reagents and protocols supplied by the DIG-labelling kit (Roche Diagnostics), except that the substrate was replaced with CDP-star (Amersham Bioscience) diluted (1 : 1) in water. Membranes were exposed to X-ray film for

2–4 h. The developed X-ray films were scanned and computer analyzed as described for dot blots.

RT-PCR

Serial dilutions (1 : 10) of total RNAs were subjected to RT-PCR using the Access RT-PCR system kit (Promega, Madison, USA) as previously described (Hourani and Abou-Jawdah, 2003) using P1: 5′ AAT AGC ATG CAA TGG CGA GTT CGA GTG AGA A 3′, and P2: 5′ AAT TCT GCA GTC AAT TAC CAC AGC CAC TG 3′. This primer pair amplifies a DNA fragment of approximately 800 bp corresponding to the CP gene.

Real-time RT-PCR SYBR Green

For RT-PCR, cDNA was synthesized in a 20 μl reaction volume using avian myeloblastosis virus (AMV) Reverse Transcriptase (Promega). PCR was performed using the primer pair (5′ TTGAAAAGGTGGGTAGGTGTTGAC 3′), and (5′ ACTCGGTGACATACGCTGGATTG 3′) to amplify a fragment of the HSP70 gene from CYSDV.

The reaction mixture (20 μl) contained AMV 1× reaction buffer, 0.5 pmole of each sense and anti-sense primers, 0.2 mM deoxynucleotide triphosphate. Mix 1 mM $MgSO_4$, 2 Units AMV Reverse Transcriptase, and 2 μl sample RNA template. The reverse transcription was carried at 50°C for 45 min followed by 94°C for 3 min.

A 10-fold serial dilution of cDNA was made and 2.5 μl of the diluted cDNA was used per reaction (25 μl). The reaction mixture contained 12.5 μl of 2× SYBR® Green supermix (Bio-Rad®), 0.5 pmole of each sense and anti-sense primers, and the volume was adjusted to 25 μl by adding nucleasefree water. Cycling parameters were evaluated to determine optimal times and temperatures for the annealing and extension steps. The optimized thermal profile was an initial denaturation step at 95°C for 3 min, followed by 31 cycles consisting each of two steps, 95°C for 15 s, and 63°C for 30s. A post-PCR melt curve was run immediately and was produced by plotting the fluorescence intensity against temperature as the temperature was increased from 55 to 95°C at 0.5°C/10 s (repeats 80). Each sample was replicated three times. Data were collected and viewed using the software and graphics programs provided with the iCycler (Bio-Rad®). Quantification was based on cDNA copy number using serial dilution of a plasmid (CYSDV HSP70 gene cloned in pGEM-T vector). The approximate molecular mass of DNA was calculated as number of bp × 650 and the CYSDV cDNA copy number was approximated as moles of plasmid × Avogadro's constant (6.02×10^{23}).

Virus movement and accumulation in tolerant and susceptible cucumber germplasms

Whiteflies reared on broccoli in insect-proof cages were transferred and allowed an access acquisition feeding period of 48 h on CYSDV-infected cucumber plants. The cucumber germplasm was kindly supplied by the United States Department of Agriculture Plant Introduction Station in Ames, IA. Cucumber seeds of tolerant accessions PI 605923, PI 293432 and PI 211589 (here referred to as 94, 57 and 29, respectively) and a susceptible accession PI 234902 (referred to as accession 49) were sown in 7-cm diameter plastic pots containing potting soil mixture. When the seedlings had the second leaf starting to emerge, 60–70 viruliferous whiteflies were collected per leaf cage and placed on the first true leaf. Whiteflies were allowed an inoculation access period of 48 h and then sprayed with Confidor® (Bayer, Leverkusen, Germany) (a.i. imidacloprid). The experiment was repeated twice in summer and fall of 2005. Leaf, petiole and root tissues were collected at 3, 5, 8, 14 and 21 dpi in the first experiment; and 4, 6, 9, 15 and 22 dpi in the second experiment from three plants. Samples originating from the same organ tissues were used for CYSDV detection using serological and nucleic acid-based methods.

Statistical analysis

Analysis was carried out with SPSS10 (Statistical Package for Social Systems).

Results

Sensitivity of serological detection methods

Detection of CYSDV by TBIA

In serial dilution assays, DBIA detected CYSDV from approximately 1 μg to 10 mg of CYSDV-infected leaf tissue. However, at the highest concentration the blots were saturated and the integrated optical density readings (IOD) did not reflect relative virus titres. Following log transformations, the effective linear range of detection varied between samples and ranged between 1 and 150 μg equivalent of CYSDV- infected tissue (Fig. 1a) with a correlation coefficient $R^2 = 0.98$ (Table 1).

Indirect ELISA

CYSDV was detected by Indirect ELISA from approximately 156 μg to 5 mg of infected tissue. Linearity of detection ranged between 156 μg and 2.5 mg infected tissue (Fig. 1b) with a correlation coefficient $R^2 = 0.98$ (Table 1).

TBIA

In cucumber leaf petioles, CYSDV was detected by TBIA in the fascicular phloem of vascular bundles, particularly in the bundle-associated cells (Fig. 2). Normally, clear CYSDV symptoms appear on susceptible cucumber germplasms approximately 3–4 weeks postinoculation (WPI). The higher the sensitivity of the detection method, the earlier it may be used to detect the virus. When a susceptible cucumber variety was inoculated with CYSDV, TBIA was the most sensitive serological method used because it allowed CYSDV detection within 5–6 dpi (Table 2); while DBIA and ELISA detected the virus 2 days later (8–9 dpi).

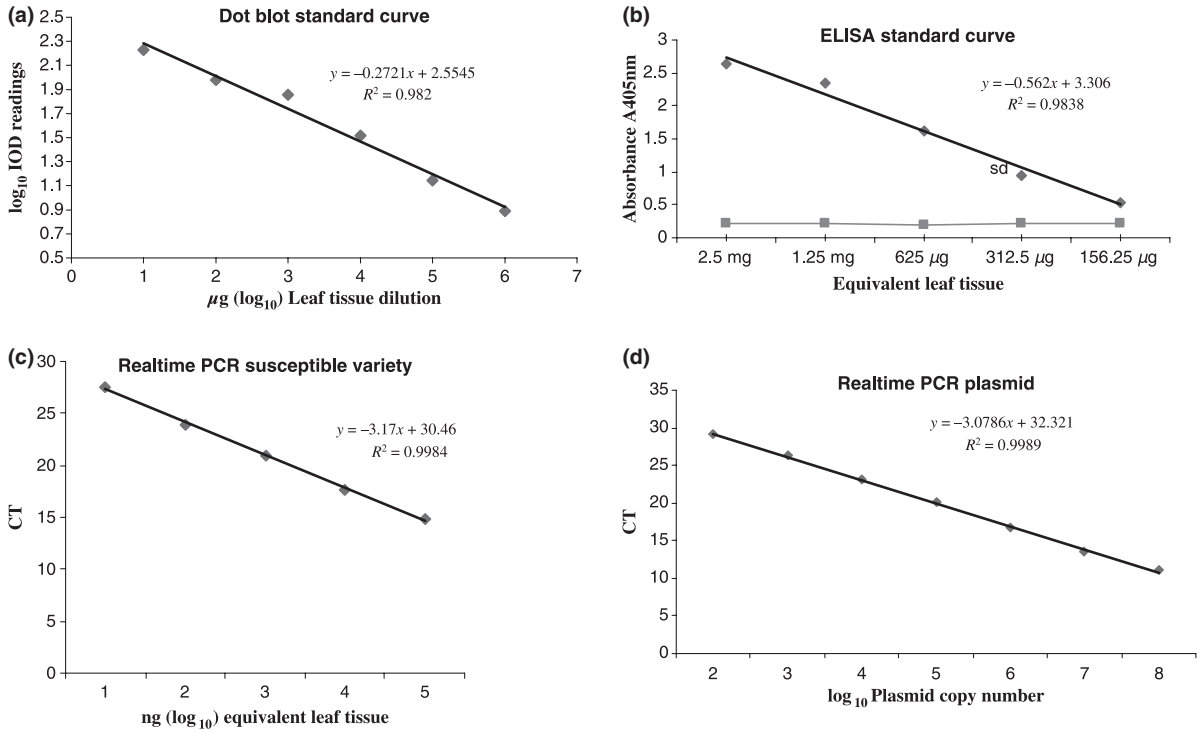

Fig. 1 Determination of the linear range of CYSDV detection using: (a) dot blot immunoassay chemiluminescent detection (Log_{10} integrated optical density readings); (b) enzyme-linked immunosorbent assay (A_{405} readings); (c) Threshold cycle (Ct) values of real-time reverese transcriptase polymerase chain reaction (RT-PCR) starting from *Cucurbit yellow stunting disorder virus* infected tissue; (d) Ct values of real-time RT-PCR starting from a recombinant plasmid

Table 1
Relative sensitivity of CYSDV detection methods

Quantity of infected leaf tissue	ELISA	DBIA[a]	NA[b] hybrid	RT-PCR	qRT-PCR[c] (Ct values)
5 mg	+	+	–	NT[d]	NT
1 mg	+	+	+	+	NT
100 μg	+	+	+	+	+(15.6)
10 μg	–	+	+	+	+(18.8)
1 μg	–	+	–	+	+(21.5)
100 ng		–	–	+	+(24.8)
10 ng		–		–	+(28.5)
Uninfected control	–	–	–	–	–NA[e]
Sensitivity compared to ELISA	1×	100×	10×	1000×	10 000×

CYSDV, *Cucurbit yellow stunting disorder virus*; ELISA, enzyme-linked immunosorbent assay;
DBIA, dot blot immunoassay; RT-PCR, reverse transcriptase polymerase chain reaction; NA, no amplification; NT, not tested; qRT-PCR, quantitative real-time RT-PCR; CDP, chemiluminescent detection.
[a]DBIA using a CDP method.
[b]Nucleic acid hybridization using a cDNA probe and a CDP method.
[c]Real-time PCR using SYBR Green, amplicon size 78 bp.
[d]Not tested.
[e]No amplification (threshold level Ct = 31).

Sensitivity of nucleic acid-based methods

Nucleic acid hybridization

Using a cDNA probe and chemiluminescent detection (CDP), CYSDV was detected in 10 μg to 1 mg CYSDV-infected tissue equivalent (Fig. 3 and Table 1) when a higher quantity (5 mg tissue equivalent) of total RNA extracts were blotted on the membrane, no signal could be detected.

RT-PCR was more sensitive than nucleic acid hybridization for detection of CYSDV. In serial dilution assays, clear amplicons were observed following gel electrophoresis of PCR amplified products from as low as 100 ng equivalent of leaf-infected tissue (Table 1).

Real-time RT-PCR using SYBR Green detected CYSDV in 10 ng tissue equivalent (Table 1). The linearity of detection ranged between 10 ng and 100 μg tissue equivalent or over four orders of magnitude with a correlation coefficient $R^2 = 0.998$ and a slope −3.199 (Fig. 1c). When plasmid carrying the CYSDV CP gene was used as a starting material, the linear range of detection reached six to seven orders of magnitude, but the best correlation coefficient was obtained over five orders of magnitude with a correlation coefficient

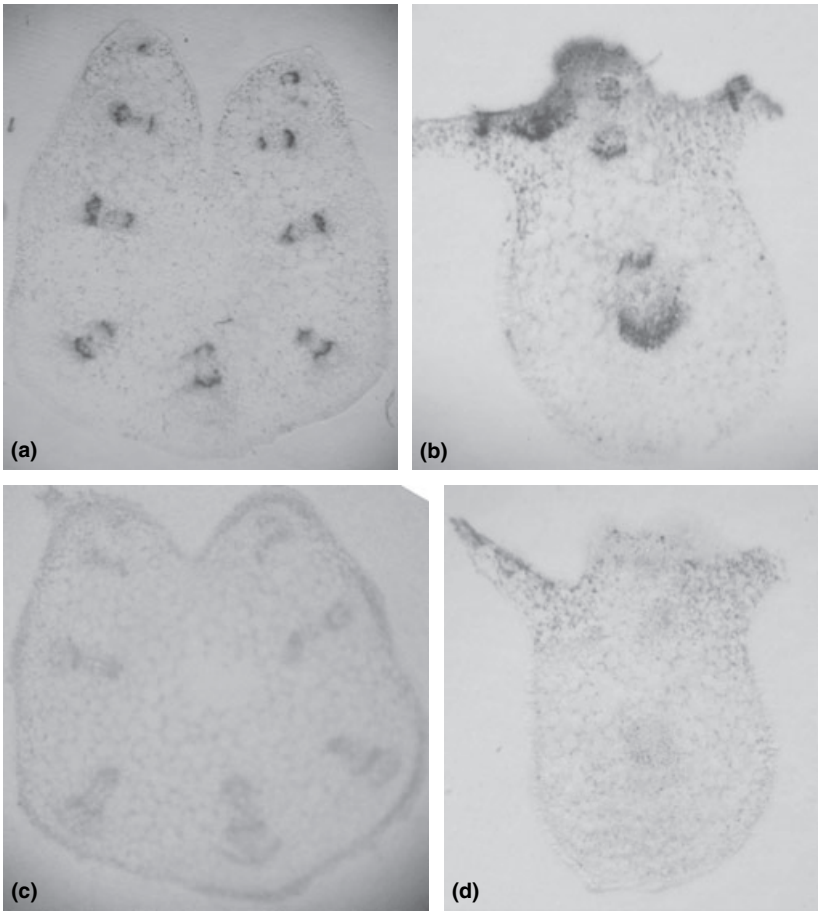

Fig. 2 Detection of *Cucurbit yellow stunting disorder virus* (CYSDV) by tissue blot immunoassay. (a) and (b) cross-sections of leaf petiole and midrib from CYSDV infected cucumber leaves; (c) and (d) their respective controls from healthy leaves

Table 2
Detection of CYSDV in the tissues of a susceptible and three tolerant cucumber accessions by Tissue Blot Immuno Assay (TBIA). Mean ratings at 5–8 and 6–9 dpi during summer and fall season

	Summer								Fall							
	49 (S)		94 (T)		57 (T)		29 (T)		49 (S)		94 (T)		57 (T)		29 (T)	
Organ	5 dpi	8 dpi	5 dpi	8 dpi	5 dpi	8 dpi	5 dpi	8 dpi	6 dpi	9 dpi	6 dpi	9 dpi	6 dpi	9 dpi	6 dpi	9 dpi
First leaf	NT	+	–	+	–	+	–	+	±	+	–	+	–	+	±	+
Second leaf	–	±	–	±	–	±	–	+	–	+	–	+	–	±	–	+
Third leaf	–	+	–	+	–	+	–	+	+	–	–	NT	±	+	–	–
Fourth leaf	–	+	NT	+	+	–	–	–	–	–	–	–	–	–	–	–
Apex	NT	+	–	+	NT	+	NT	+	–	+	–	+	–	±	–	+
Root	+	+	–	+	±	+	+	+	±	+	–	+	–	+	±	+
Negative	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

CYSDV, *Cucurbit yellow stunting disorder virus*; NT, not tested.
+: Clear precipitate indicating relatively high CYSDV concentration.
±: Faint precipitate indicating relatively low CYSDV concentration.
–: No detection.

R^2 = 0.998 and a slope −3.078 (Fig. 1d). The lowest limit of detection was 110 copies of the CYSDV cDNA.

CYSDV movement at early stages of infection

The virus movement and titre were monitored following inoculation of first true cucumber leaf with CYSDV using whiteflies and one susceptible (49) and three tolerant (94, 57, 29) accessions. In the susceptible accession, CYSDV could not be detected by any of the detection methods at 3 dpi. However, at 5–6 dpi, the virus was detected in the root system and the inoculated leaf by real-time RT-PCR. Similarly, TBIA could detect the virus in the root tissue 5–6 dpi (Table 2). At 8–9 dpi, CYSDV was detected in all sampled tissues by real-time RT-PCR, TBIA and DBIA. At this stage, the intensity of colorimetric detection by TBIA was higher in the root and crown tissues than in inoculated leaves, apex, and second and third leaves. At 14 and 21 dpi, CYSDV was also detected by TBIA in all

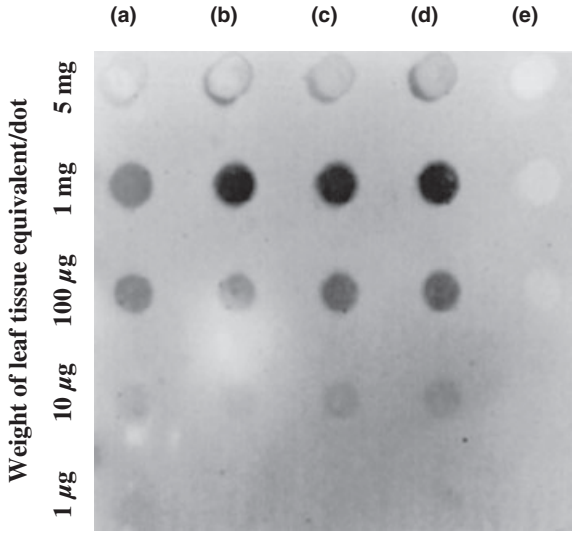

Fig. 3 Detection of *Cucurbit yellow stunting disorder virus* (CYSDV) by nucleic acid hybridization using a cDNA probe and chemiluminescent detection system. (a), (b), (c) and (d) are extracts of CYSDV-infected cucumber leaves; the right lane represents an extract of a healthy leaf. Detection was possible between 10 μg and 1 mg equivalent of CYSDV-infected leaf tissue

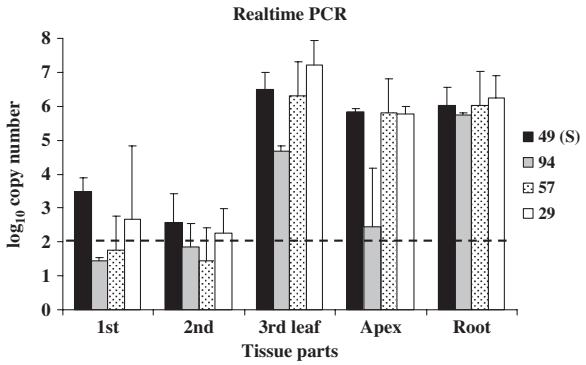

Fig. 4 Comparison of *Cucurbit yellow stunting disorder virus* (CYSDV) concentrations expressed as cDNA copy numbers in tissue extracts of a susceptible (49) and three tolerant (94, 57 and 29) cucumber accessions using real-time reverse transcriptase polymerase chain reaction (RT-PCR) at 8 dpi. First to third leaf represents the inoculated leaf and the order of leaves above it, respectively. The horizontal dotted line denotes the normal limit of detection; for calculation purposes a Ct value of 31 was given for samples that were negative by real-time RT-PCR

tested tissues. The same pattern was observed in tolerant accessions 29 and 57. However, in the most tolerant accession 94, a slight delay in CYSDV detection was observed and virus was not detected 5–6 dpi and was detected in all tissues samples 8–9 dpi (Table 2). These data strongly suggest that virus movement is not the primary factor governing tolerance to CYSDV in these accessions.

CYSDV titre at early stages of infection

Relative CYSDV concentrations were compared in different organs of tolerant and susceptible accessions using real-time RT-PCR at 8 dpi. CYSDV was detected in all tissues sampled. No significant differences in CYSDV concentrations were detected in the root system of all four accessions. CYSDV concentration was close to 5×10^5 copies of CYSDV cDNA in all accessions. Low CYSDV titres, at the limit of detection (approximately 110–1100 copies of CYSDV cDNA) were observed in the inoculated leaf and the leaf above it in all four accessions. However, the highly tolerant accession 94 showed lower virus concentration in the third leaf (4.6×10^4 copies of CYSDV cDNA) and in the apex tissue (2.6×10^2 copies of CYSDV cDNA) as compared to those of the susceptible (3×10^6 and 7×10^5 copies of CYSDV cDNA) in the third leaf and apex, respectively (Fig. 4). CYSDV concentration in tissues of the other two tolerant accessions 57 and 29 were close to those of the susceptible control.

DBIA results at 9 dpi showed that in the susceptible, CYSDV was detected in all tissues tested in the inoculated leaf (first leaf), second, and third leaf, and in the apex; with the highest CYSDV concentration in the root system. In the tolerant accession 94, CYSDV concentration in the root system was similar to that of

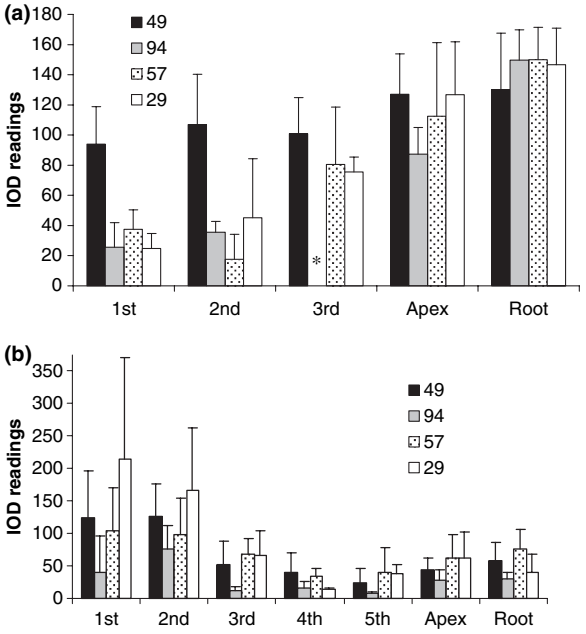

Fig. 5 Comparison of relative *Cucurbit yellow stunting disorder virus* (CYSDV) concentrations in tissue extracts of a susceptible (49) and three tolerant (94, 57 and 29) cucumber accessions using dot blot immunoassay chemiluminescent detection. Integrated optical density readings at 9 dpi (a) and 22 dpi (b). First to fifth leaf represents the inoculated leaf and the order of leaves above it, respectively. *Accession 94 did not develop a third leaf at this sampling time of the fall experiment

the susceptible, but slightly lower in the first and second leaf and in the apex. In the third leaf, the IOD readings were 101 ($\pm$24), 80 ($\pm$38) and 75 ($\pm$10) for the susceptible and the tolerant accessions 29 and 57, respectively; while accession 94 did not bear a third leaf (Fig. 5). AT 22 dpi, CYSDV was detected by DBIA in the roots, first, second and third, fourth and fifth leaves, and the apex of tolerant accession 94. However, CYSDV concentrations in all these tissues were lower

than in the respective tissues of the susceptible as well as most tissues of the other two tolerant accessions. CYSDV concentrations at 22 dpi in most studied tissues of the other two tolerant accessions (29 and 57) were closer or similar to those of the susceptible.

It is worth mentioning that statistical analysis of CYSDV concentration using any detection method was difficult because variations in response to CYSDV infection were observed between plants, such variations were previously reported to occur among individual plants of tolerant varieties (Marco et al., 2003; Eid et al., 2006).

Discussion

In Lebanon and other countries, cucumber seeds are sown in specialized nurseries and seedlings are transported to farmers' fields in distant areas for transplanting. Fast, sensitive, simple and reliable virus detection methods are an important tool in the management of virus diseases. Evaluation and optimization of detection methods are a prerequisite for their use. In our study, the sensitivity of techniques used for CYSDV detection was compared based on their ability to detect the virus in serial dilutions of extracts from infected leaf tissue. Real-time RT-PCR was the most sensitive detection method followed by RT-PCR, DBIA, nucleic acid hybridization and ELISA which allowed CYSDV detection in a minimum of 10 ng, 100 ng, 1–10 μg; 10 μg and 100 μg of CYSDV-infected tissue equivalents, respectively.

Symptoms caused by CYSDV appear on the lower leaves of susceptible cucumber approximately 3 WPI and become more obvious approximately 1 week later. Approximately 1 WPI, the highest CYSDV concentration was detected in roots using serological detection, making this the preferred tissue for virus detection. However, in approximately 2–3 WPI, relative CYSDV concentrations declined in the roots and increased in the leaves. These results are slightly different from those reported by Marco et al. (2003). Using nucleic acid hybridizations, they were able to detect CYSDV in a susceptible cucumber only in the two leaves above the inoculated one at 1 and 2 WPI, and that the virus could not be detected after 3 or 4 WPI in any leaf tissue of cucumber, melon, marrow or squash.

In a previous study (Hourani and Abou-Jawdah, 2003), we showed that TBIA is a fast, sensitive and reliable technique for CYSDV detection in cucumber leaf petioles. In this study, TBIA allowed CYSDV detection within 5–6 dpi in the susceptible accession 49. Using DBIA or ELISA, CYSDV detection was only possible 8–9 dpi. Using real-time RT-PCR CYSDV was not detected at 3 dpi, but was detected at 5 dpi in the root system and the inoculated leaf. Therefore, for practical applications, TBIA is recommended for rapid detection and for comparison of CYSDV movement between resistant and susceptible cucumber accessions.

Resistance to CYSDV in melon accession C-105 was correlated with strongly reduced virus accumulation (Marco et al., 2003). Likewise, resistance to *Cucumber mosaic virus* in cucumber cv. Delila was correlated with low levels of virus RNA accumulation (Wang et al., 2003), while resistance in soybean PI 346304 to *Cowpea chlorotic mottle virus* and resistance of tomato genotypes carrying the *Ty-1* gene to *Tomato yellow leaf curl virus* were related to restriction or inhibition of virus movement (Goodrick et al., 1991; Michelson et al., 1994). Data of a previous study (Eid et al., 2006) demonstrated that CYSDV-tolerant germplasms showed delayed symptom expression and the proportion of diseased plants were much lower than for susceptible accessions. The tolerance of the three cucumber accessions used in this study, as evaluated by TBIA was not correlated with a delay or restriction of virus movement. As TBIA is based on the detection of CP, the serological detection methods were complemented with nucleic acid-based methods to reveal that the CYSDV RNA is present in tissues where the CP was also detected. Furthermore, preliminary results using DBIA and real-time RT-PCR-based assays proved that tolerance to CYSDV in two accessions (29 and 57) was not correlated with reduced virus accumulation. This tolerance is probably similar to that reported for commercial '*Zucchini yellow mosaic virus* (ZYMV)-resistant' zucchini cultivars, where ZYMV induces very mild symptoms but virus multiplication remains normal (Lecoq et al., 2004). The higher tolerance of accession 94 may be correlated with reduced virus accumulation in young leaves, but not in roots. Apparently, accessions 29, 57 and 94 may tolerate higher virus concentrations before development of symptoms. Accession 94 may have a mechanism that suppresses virus accumulation in young leaves more efficiently than the other tolerant accessions or the susceptible control.

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